

## Short communication

*Toxoplasma gondii* infection in Blanford's fox (*Vulpes cana*)J.P. Dubey<sup>a,\*</sup>, An Pas<sup>b</sup><sup>a</sup> United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute,  
Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA<sup>b</sup> Breeding Centre for Endangered Arabian Wildlife, PO Box 29922, Sharjah, United Arab Emirates

Received 19 September 2007; received in revised form 14 January 2008; accepted 17 January 2008

---

**Abstract**

Fatal toxoplasmosis was diagnosed in a Blanford's fox (*Vulpes cana*) from the United Arab Emirates. *Toxoplasma gondii*-like tachyzoites were found associated with necrosis in intestine, spleen, liver, kidneys, lungs, skeletal muscle, brain and heart. Protozoal tachyzoites reacted positively with *T. gondii*-specific polyclonal antibodies. Antibodies to *T. gondii* were detected in 10 of 12 *V. cana* assayed by the latex agglutination or the modified direct agglutination test.

Published by Elsevier B.V.

**Keywords:** *Toxoplasma gondii*; Blanford's fox (*Vulpes cana*); Fatal toxoplasmosis; Antibodies; United Arab Emirates

---

**1. Introduction**

*Toxoplasma gondii* infections are widely prevalent in humans and other animals worldwide (Dubey and Beattie, 1988). Fatal toxoplasmosis has been reported in several species of foxes including the red fox (*Vulpes vulpes*), the arctic fox (*Vulpes lagopus*), and the gray fox (*Urocyon cinereoargenteus*) (Møller and Nielsen, 1964; Dubey and Beattie, 1988; Dubey and Lin, 1994; Sørensen et al., 2005). Viable *T. gondii* has been isolated from a *V. vulpes*, an arctic fox (*Vulpes lagopus*) (Prestrud et al., 2008) and a *U. cinereoargenteus* (Dubey et al., 2004). We report fatal toxoplasmosis in a Blanford's fox (*Vulpes cana*), first time from this host.

The Blanford's fox is a small animal weighing between 1 and 1.5 kg with a distinctive long, bushy tail which can be almost the same length as the body. They occur over a disjointed range in Arabia, including Oman

and Saudi Arabia, as well as north east Iran, Afghanistan, Central Asia, India and Pakistan. Its presence in the UAE was only confirmed in 1995 (Hellyer and Aspinall, 2005).

Due to rapid human development their habitat is decreasing and in some areas they have been heavily hunted for their fur (Sillero-Zubiri et al., 2004). The Blanford's fox is now listed under Cites II regulations (Sillero-Zubiri et al., 2004). These nocturnal animals feed in the wild on invertebrates, reptiles, small mammals and fruit (Hellyer and Aspinall, 2005). In captivity they reach sexual maturity at about 1 year of age and the females can give birth to a litter of 2–4 pups after a gestation period of 51–55 days. The litter will be suckled for 6–8 weeks after which they will be weaned and eat the same diet as their parents.

**2. Materials and methods***2.1. Naturally exposed foxes*

A small group of *V. cana* were kept at the Breeding Centre for Endangered Arabian Wildlife (BCEAW),

---

\* Corresponding author. Tel.: +1 301 504 8128;  
fax: +1 301 504 9222.

E-mail address: [jitender.dubey@ars.usda.gov](mailto:jitender.dubey@ars.usda.gov) (J.P. Dubey).

Sharjah, United Arab Emirates. The foxes at the BCEAW are kept in pairs together with their offspring, and were fed rats and mice from in-house breeding facility, fruit and vegetables, chicken, day old chicks, buffalo, beef, camel and donkey meat depending on availability. Insects (locust, crickets, mealworms) are added as enrichment more than as a significant portion of their diet.

## 2.2. Index case

In March 2006 a litter of three Blanford's fox pups, two males and one female (VC038, VC039, VC040) was born at BCEAW. The dam (VC009) had been wild caught as a juvenile and was now about 8 years old. She had successfully raised several litters over the years. When the pups were about 6 weeks old the dam sustained a compound fracture and was euthanized because of poor prognosis and age of the animal. The male (VC006) was still with the pups and would provide protection. The pups were slowly weaned.

Around the age of 10 weeks all 3 pups developed skin problems. They had marked areas of alopecia and some crust formation spread out over the body which were indicative for ringworm. Pups were medicated topically with betadine (Claradone, Povidone–Iodine USP 10%, Medpharma, Sharjah, UAE) every 48 h. Only a few days later one pup (VC038) was found dead in the morning. The 2 other pups recovered well from their skin infection, never showed signs of illness and gained weight at a normal rate.

### 2.2.1. Histopathological examination

The pup was examined at necropsy. Samples of spleen, kidneys, heart, intestine, brain, liver, lungs, and

skeletal muscle were fixed in 10% formalin. Formalin-fixed tissues were sent to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, MD for diagnosis. Tissues were processed routinely for paraffin embedding and sectioning. After staining with hematoxylin and eosin (HE), sections were examined microscopically.

### 2.2.2. Immunohistological examination

At APDL, paraffin-embedded tissue sections were reacted with antibodies to *T. gondii* and *Neospora caninum* polyclonal rabbit antibodies as described (Lindsay and Dubey, 1989; Dubey et al., 2001). In addition sections were reacted with bradyzoite-specific *T. gondii* BAG 1 antibodies (McAllister et al., 1996). The polyclonal *T. gondii* antibodies react with both tachyzoites and bradyzoites of *T. gondii* and not *N. caninum* whereas the BAG 1 antibody reacts with bradyzoites and not tachyzoites but is not specific for *T. gondii* or *N. caninum*.

### 2.3. Serological examination for *T. gondii*

Serum from all the Blanford's foxes at the BCEAW banked frozen at  $-20^{\circ}\text{C}$  in previous years (Table 1) was retrospectively tested for antibodies to *T. gondii* using the modified agglutination test (MAT) and the latex agglutination test (LAT). The MAT was performed at the APDL as described by Dubey and Desmonts (1987) using 1:25 to 1:3200 dilutions. The LAT was performed at the University of Glasgow, Scotland, using a commercial kit (Eiken, Tokyo, Japan); a titer of 1:64 was used as a cut-off value.

Table 1  
Antibodies to *T. gondii* in sera of Blanford's foxes

Samples (month/day/year)	ID, sex	Source	Date of birth	MAT	LAT
9/10/1998	VC001, F <sup>a</sup>	Wildcaught 1997	1996	Not done	270
9/10/1998	VC002, F	Wildcaught 1997	1997	Not done	7290
9/10/1998	VC003, M	Wildcaught 1997	1996	Not done	2430
9/10/1998	VC005, M	Captive born	4/10/1998	Not done	<64
11/13/2006	VC005, M	Captive born	4/10/1998	>3200	Not done
11/9/2006	VC006, M	Wildcaught 1999	1997	>3200	Not done
7/16/2002	VC007, F	Wildcaught 1999	1997	>3200	Not done
11/13/2006	VC008, F	Wildcaught 1999	1998	>3200	Not done
5/20/2003	00 060D 5FDE, M	Wildcaught and released	Unknown	<25	Not done
4/8/2003	00 060F 1083, M	Wildcaught and released	Unknown	<25	Not done
6/22/2006	VC026, M	Captive born	3/22/2004	>3200	Not done
7/30/2006	VC039, M	Captive born	3/15/2006	>3200	Not done
7/30/2006	VC040, M	Captive born	3/15/2006	>3200	Not done

<sup>a</sup> F, female; M, male.

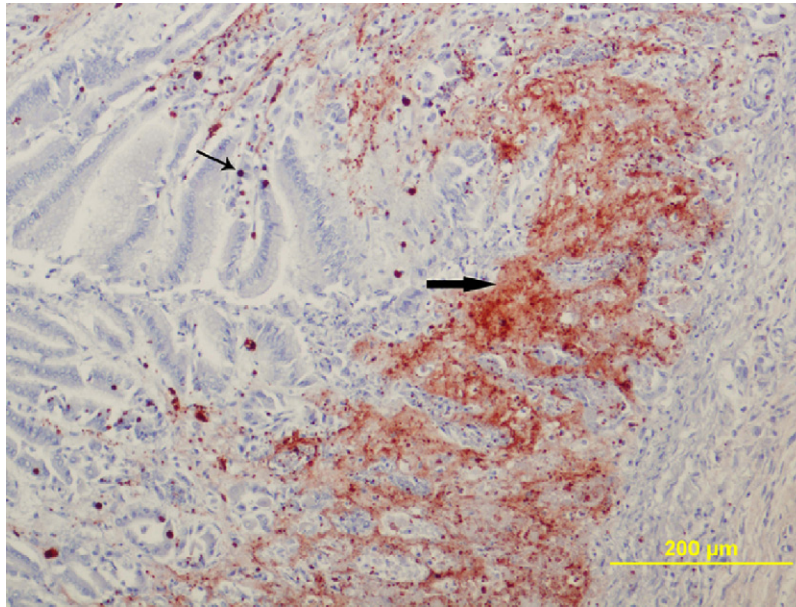


Fig. 1. Section of small intestine showing numerous tachyzoites (all red areas) in the lamina propria (large arrow) and few tachyzoites apparently in enterocytes (small arrow). Immunohistochemical staining with polyclonal *T. gondii* antibodies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

### 3. Results

#### 3.1. Index case

Gross lesions were found in lungs and liver. The lungs were edematous, the liver was icteric and swollen,

and serohemorrhagic fluid was present in the thorax and abdominal cavity.

Microscopically, lesions were present in the spleen, liver, heart, intestine, kidneys, and lungs. The intestinal lesions were characterized by necrosis of lamina propria and many tachyzoites were present

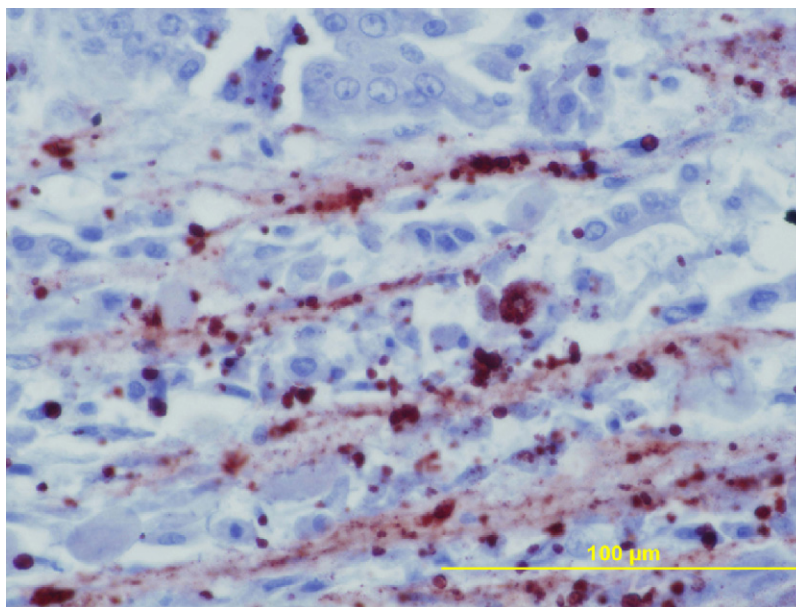


Fig. 2. Section of small intestinal lamina propria, showing numerous tachyzoites (all red areas). Immunohistochemical staining with polyclonal *T. gondii* antibodies. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

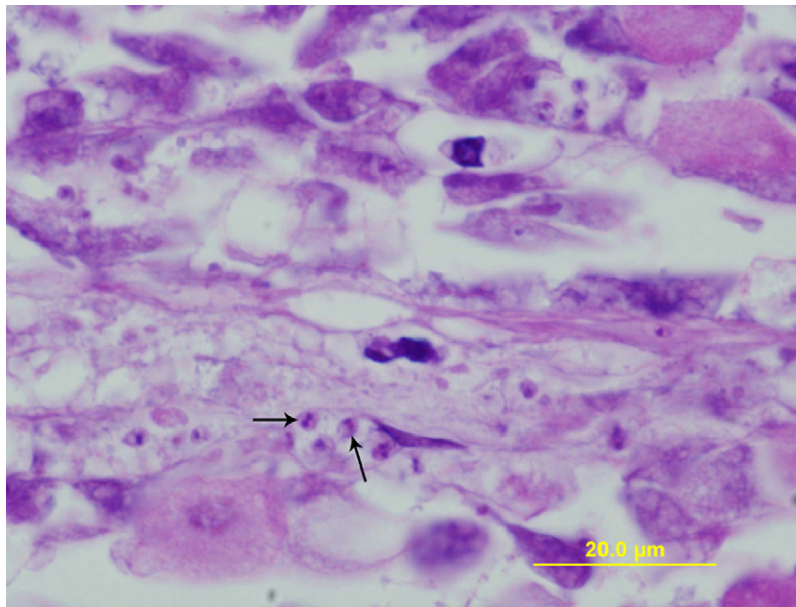


Fig. 3. Section of intestinal lamina propria showing necrosis and several individual tachyzoites, some of them are dividing (arrows). Hematoxylin and eosin stain.

in cells of the lamina propria; a few tachyzoites were also seen in enterocytes (Figs. 1 and 2). In HE-stained sections only a few organisms were visible, even under the oil immersion (Fig. 3). In spleen there was lymphoid cell depletion and numerous individual tachyzoites were present in necrotic areas. Multiple foci of hepatic necrosis were associated with tachyzoites. Few necrotic foci were seen in the renal intratubular areas, in skeletal muscle, and the myocardium; tachyzoites were present in lesions. The pulmonic lesions consisted of edema, infiltration of macrophages in alveoli, and focal bronchitis. A small focus of gliosis was present in cerebrum and a group of tachyzoites was present at the periphery of the cerebral lesion.

Protozoa reacted positively to *T. gondii* polyconal antibodies but not with BAG-1 *T. gondii* antibodies and *N. caninum* polyclonal antibodies. Immunohistochemical test performed at a commercial laboratory was negative for Distemper Virus Infection.

Fluid from the abdomen and thorax was cultured at the Nad Al Sheba Veterinary Hospital, Dubai, UAE, but no significant bacterial growth was seen.

### 3.2. Serological examination

In total there were 12 foxes tested. Antibodies to *T. gondii* were not found in two wild caught foxes that were bled and released. Antibodies to *T. gondii* were

found in 10 of 10 captive foxes (Table 1). Serum samples were not available from the index case fox and its dam.

## 4. Discussion

The present index case was diagnosed as primary acute toxoplasmosis based on finding of extensive lesions in the intestine and liver, absence of bradyzoites, and other concurrent infections. The etiological diagnosis was confirmed by immunohistochemical examination. Primary toxoplasmosis in foxes is rare and has been reported once in a red fox (Dubey et al., 1990), and a gray fox (Dubey and Lin, 1994). Other reports of fatal toxoplasmosis in red fox (Helmboldt and Jungherr, 1955; Moller and Nielsen, 1964; Reed and Turek, 1985; Kelly and Sleeman, 2003), silver fox (Moller, 1952), and gray fox (Kelly and Sleeman, 2003) were considered associated with Canine Distemper Virus (CDV) infection. The CDV infection is immunosuppressive and most cases of toxoplasmosis in dogs are as a complication of CDV infection (Campbell et al., 1955; Dubey et al., 2003). In the present case there was no evidence for CDV infection. Sørensen et al. (2005) reported fatal toxoplasmosis in three arctic foxes on Svalbard in the absence of CDV infection; one of these foxes had a co-infection with *Yersinia pseudotuberculosis* and *Salmonella enteritidis* that can be linked to immunosuppression. Kottwitz et al. (2004) reported primary toxoplasmosis in a captive Fennec fox (*Fennecus zerda*).



The character of lesions in the fox in the present study, indicates that the fox acquired *T. gondii* infection recently, probably within two weeks of death. The fox had severe necrosis in intestines, spleen and liver associated with many tachyzoites. The lesion in the brain was small in size and had only a few tachyzoites. After the ingestion of *T. gondii* tissue cysts or oocysts in experimental infections, tachyzoites first multiply in the intestinal lamina propria and visceral tissues. During the second week after infection tachyzoites begin to colonize the brain (Dubey and Frenkel, 1973; Dubey and Beattie, 1988). Some animals can die of enteritis and mesenteric lymph node necrosis before other tissues are diseased. Bradyzoites are formed in tissues starting the first week after ingestion of *T. gondii* (Dubey, 1997; Dubey et al., 1997). In the present case, bradyzoites were not found in sections of any tissues, supporting the diagnosis of recently acquired infection.

Serological results indicate that not all foxes exposed to *T. gondii* become ill. It is likely that the foxes in the present study became infected at BCEAW after eating tissues of animals infected with *T. gondii*. After the confirmation of *T. gondii* infection in captive foxes the diet has been changed. Now, captive animals at BCEAW are fed only frozen meat and rodents bred at BCEAW.

## Acknowledgement

We thank O.C.H. Kwok for performing serological and immunohistochemical examinations.

## References

- Campbell, R.S.F., Martin, W.B., Gordon, E.D., 1955. Toxoplasmosis as a complication of canine distemper. *Vet. Rec.* 67, 708–716.
- Dubey, J.P., 1997. Bradyzoite-induced murine toxoplasmosis: stage conversion, pathogenesis, and tissue cyst formation in mice fed bradyzoites of different strains of *Toxoplasma gondii*. *J. Eukaryot. Microbiol.* 44, 592–602.
- Dubey, J.P., Beattie, C.P., 1988. *Toxoplasmosis of Animals and Man*. CRC Press, Boca Raton, Florida, pp. 1–220.
- Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. *Equine Vet. J.* 19, 337–339.
- Dubey, J.P., Frenkel, J.K., 1973. Experimental *Toxoplasma* infection in mice with strains producing oocysts. *J. Parasitol.* 59, 505–512.
- Dubey, J.P., Lin, T.L., 1994. Acute toxoplasmosis in a gray fox (*Urocyon cinereoargenteus*). *Vet. Parasitol.* 51, 321–325.
- Dubey, J.P., Hamir, A.N., Rupprecht, C.E., 1990. Acute disseminated toxoplasmosis in a red fox (*Vulpes vulpes*). *J. Wildlife Dis.* 26, 286–290.
- Dubey, J.P., Speer, C.A., Shen, S.K., Kwok, O.C.H., Blixt, J.A., 1997. Oocyst-induced murine toxoplasmosis: life cycle, pathogenicity, and stage conversion in mice fed *Toxoplasma gondii* oocysts. *J. Parasitol.* 83, 870–882.
- Dubey, J.P., Garner, M.M., Willette, M.M., Batey, K.L., Gardiner, C.H., 2001. Disseminated toxoplasmosis in magpie geese (*Anseranas semipalmata*) with large numbers of tissue cysts in livers. *J. Parasitol.* 87, 219–223.
- Dubey, J.P., Ross, A.D., Fritz, D., 2003. Clinical *Toxoplasma gondii*, *Hammondia heydorni*, and *Sarcocystis* sp. infections in dogs. *Parassitologia* 45, 141–146.
- Dubey, J.P., Graham, D.H., de Young, R.W., Dahl, E., Eberhard, M.L., Nace, E.K., Won, K., Bishop, H., Punkosdy, G., Sreekumar, C., Vianna, M.C.B., Shen, S.K., Kwok, O.C.H., Summers, J.A., Demarais, S., Humphreys, J.G., Lehmann, T., 2004. Molecular and biologic characteristics of *Toxoplasma gondii* isolates from wildlife in the United States. *J. Parasitol.* 90, 67–71.
- Hellyer, P., Aspinall, S. (Eds.), 2005. *The emirates. A natural history*. Trident Press Ltd., Mayfair, London, UK, p. 312.
- Helmholtz, C.F., Jungherr, E.L., 1955. Distemper complex in wild carnivores simulating rabies. *Am. J. Vet. Res.* 16, 463–469.
- Kelly, T.R., Sleeman, J.M., 2003. Morbidity and mortality of red foxes (*Vulpes vulpes*) and gray foxes (*Urocyon cinereoargenteus*) admitted to the Wildlife Center of Virginia, 1993–2001. *J. Wildlife Dis.* 39, 467–469.
- Kottwitz, J.J., Preziosi, D.E., Miller, M.A., Ramos-Vara, J.A., Maggs, D.J., Bonagura, J.D., 2004. Heart failure caused by toxoplasmosis in a fennec fox (*Fennecus zerda*). *J. Am. Anim. Hosp. Assoc.* 40, 501–507.
- Lindsay, D.S., Dubey, J.P., 1989. Immunohistochemical diagnosis of *Neospora caninum* in tissue sections. *Am. J. Vet. Res.* 50, 1981–1983.
- McAllister, M.M., Parmley, S.F., Weiss, L.M., Welch, V.J., McGuire, A.M., 1996. An immunohistochemical method for detecting bradyzoite antigen (BAG5) in *Toxoplasma gondii*-infected tissues cross-reacts with a *Neospora caninum* bradyzoite antigen. *J. Parasitol.* 82, 354–355.
- Moller, T., 1952. Toxoplasmosis *Vulpis vulpis* (Fox toxoplasmosis). *Acta Pathol. Microbiol. Scand.* 93 (Suppl.), 308–320.
- Moller, T., Nielsen, S.W., 1964. Toxoplasmosis in distemper-susceptible carnivora. *Pathol. Vet.* 1, 189–203.
- Prestrud, K.W., Dubey, J.P., Åsbakk, K., Fuglei, E., Su, C., 2008. First isolate of *Toxoplasma gondii* from arctic fox (*Vulpes lagopus*) from Svalbard. *Vet. Parasitol.* 151, 110–114.
- Reed, W.M., Turek, J.J., 1985. Concurrent distemper and disseminated toxoplasmosis in a red fox. *J. Am. Vet. Med. Assoc.* 187, 1264–1265.
- Sillero-Zubiri, C., Hoffmann, M., MacDonald, D.W. (Eds.), 2004. *Canids: Foxes, Wolves, Jackals and Dogs. Status Survey and Conservation Action Plan*. IUCN/SSC Canid Specialist Group, Gland, Switzerland and Cambridge, UK, pp. 194–198.
- Sørensen, K.K., Mørk, T., Siguroardóttir, Ó.G., Åsbakk, K., Åkersed, J., Bergsjø, B., Fuglei, E., 2005. Acute toxoplasmosis in three wild arctic foxes (*Alopex lagopus*) from Svalbard; one with co-infections of *Salmonella enteritidis* PT1 and *Yersinia pseudotuberculosis* serotype 2b. *Res. Vet. Sci.* 78, 161–167.